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PRIMER NOTE

PCR primers for trinucleotide and tetranucleotide microsatellites in greater amberjack, *Seriola dumerili*

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Abstract

Eighteen nuclear-encoded microsatellites from a genomic DNA library of greater amberjack, *Seriola dumerili*, were isolated and characterized. The microsatellites include 13 perfect (five tetranucleotide and eight trinucleotide) and five imperfect (three tetranucleotide, one trinucleotide and one combination dinucleotide/trinucleotide) repeat motifs. The number of alleles at the 18 microsatellites among a sample of 29 fish ranged from two to 20; gene diversity (expected heterozygosity) ranged from 0.068 to 0.950, whereas observed heterozygosity ranged from 0.069 to 0.966. Following Bonferroni correction, genotypes at all 18 microsatellites fit expectations of Hardy–Weinberg equilibrium, and all pairwise comparisons of microsatellites did not deviate significantly from genotypic equilibrium. Greater amberjack support commercial and recreational fisheries along both the Atlantic and the Gulf coasts of the USA and represent a species with potential for worldwide aquaculture. The microsatellites developed will be useful for population genetic studies of 'wild' populations and breeding studies of domesticated populations.

Keywords: genomic library, greater amberjack, microsatellites, *Seriola dumerili*

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Greater amberjack, *Seriola dumerili*, is an economically important, reef-associated carangid fish with a circumglobal distribution in subtropical and temperate waters (Manooch & Potts 1997). In U.S. waters, increased commercial and recreational fishing pressure on this species has led to the establishment of fishery limitations along both the Atlantic and Gulf coasts (NMFS 2005; SAFMC 2005). Additionally, there is worldwide interest in commercial aquaculture of greater amberjack (Mylonas *et al*. 2004) due to its relatively rapid growth rate and excellent market potential.

In this note, we report development of polymerase chain reaction (PCR) primers for 18 nuclear-encoded microsatellites from a greater amberjack genomic DNA library. Nuclear-encoded microsatellites are well suited for (i) providing population genetic data to assist with management of 'wild' stocks (Gold & Richardson 1998); and (ii) application in aquaculture investigations, including assessment of genetic variability and inbreeding, parentage assignment and quantitative trait loci (QTL) identification (Liu & Cordes 2004).

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Whole genomic DNA was extracted from ethanol-preserved muscle tissue, using a standard phenol–chloroform method; two separate digestions were performed with *Dpn*II and *Apo*I (New England BioLabs). Size-selected fragments (750–1500 bp) were ligated into a *Bam*HI- or *Eco*RI- (New England BioLabs) digested and dephosphorylated (Calf Intestinal Alkaline Phosphatase, New England BioLabs) pBluescript vector (Stratagene), using T4 DNA ligase (New England BioLabs), and transformed into XL10-Gold Ultracompetent cells (Stratagene). Transformed cells were plated on X-Gal/IPTG Luria-Bertani (LB) agar with 100 μg/mL of ampicillin and grown overnight at 37 °C. Recombinant colonies were picked using a Genetix Q-BOT, inoculated into 384-well plates that contained 50 μL of LB freezing media [36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM sodium citrate, 0.4 mM $MgSO_4$, 6.8 mM $(NH_4)_2SO_4$, 4.4% (v/v) glycerol, 100 μg/mL ampicillin, LB], and incubated overnight at 37 °C before freezing at –80 °C.

A total of 18 432 clones (48 × 348-well plates), 10 368 from *Apo*I digests (27 × 384-well plates) and 8064 from

Table 1 Summary data for microsatellites developed from the greater amberjack, *Seriola dumerili*

Msat	GenBank	Primer sequence (5′–3′)	Repeat sequence	T_a	N/N_a	Size range	H_E/H_O	P_{HW}
Sdu 1	DQ468081	**CGTTTCCATCGCACTTTT***** GCTAACACTCACTGGTG	$(GACA)_{20}$	53	28/11	305–385	0.751/0.714	0.252
Sdu 2	DQ468082	**CTATATTCACTCTGTTGCC**** GTGTAGGAGAGACTGTAAG	$(GAA)_8$	50	29/5	145–160	0.716/0.670	0.918
Sdu 3	DQ468083	**CGGTGTATTGTTACTGTGAC*** TCGTCTCTGATTGGTTAG	$(CAA)_8$	53	29/5	212–227	0.602/0.690	0.717
Sdu 4	DQ468084	**GGAAATAGTTTGGATCACGCTGG***** GGATGCTCAGTGAAGTTGTGC	$(GACA)_8(GGCA)_4(GACA)_6$	60	29/10	320–360	0.808/0.897	0.972
Sdu 5	DQ468085	**GTAAGGATTTGTCATGTAGCC**** GGAGACGAGTTCTCTTTGC	$(TAA)_3CAA(TAA)_{10}CAA(TAA)_5$	56	23/5	206–236	0.560/0.478	0.390
Sdu 6	DQ468086	**CCAAAGCAGGTGAAAGTGA*** GGTCCATACAACAACTCAG	$(GATA)_{12}$	50	29/12	231–279	0.844/0.759	0.043
Sdu 7	DQ468087	**CACTTTCAACTGGAACACC**** GGTTCTGCTGGCTCATTG	$(CAA)_8$	56	26/3	343–364	0.361/0.269	0.102
Sdu 8	DQ468088	**CCAGTCTATGAAACACAACC*** CCTGAAGCGATGAAGCGT	$(GAA)_9$	56	29/3	105–111	0.068/0.069	1.000
Sdu 9	DQ468089	**CTGTTGTCCTTCCAGAC***** CCACATCGTCTGAATAGC	$(GA)_4(GAA)_9$	53	29/2	230–238	0.131/0.138	1.000
Sdu 10	DQ468090	**CCAAGTCCTCCTGCTACTACCAT*** CCTTGTGGATGACCTGTTTG	$(GAA)_{18}$	56	29/15	295–346	0.902/0.966	0.412
Sdu 11	DQ468091	**GCTCTCGTGTGTTACTCAAG*** GCAACTGTCAGATCCTCCA	$(CAA)_7$	56	29/2	169–172	0.160/0.172	1.000
Sdu 12	DQ468092	**CCACAAGTTATCACAAGCCACC**** GCTTTGTCCCCTGTGTGCTG	$(GACA)_5GGCA(GACA)_5GGCA(GACA)_7$	60	29/10	237–313	0.776/0.690	0.550
Sdu 16	DQ468093	**GAGTTGTACTGTGGTAAAC*** GGACATTAGAGTCTGTGG	$(CAA)_{11}$	50	29/4	114–126	0.492/0.552	0.174
Sdu 19	DQ468094	**GCATTCTGGCATTAGCAT***** GGTACTCTAGTTAGCCCTAC	$(CAGA)_{16}$	56	29/10	236–272	0.823/0.724	0.178
Sdu 21	DQ468095	**CTCAGGACAATGTTGGTAG*** GCTAACAAGTTCACGACAT	$(GATA)_{25}$	56	27/20	264–380	0.950/0.926	0.697
Sdu 22	DQ468096	**CATTCTCCAAGTATGTGACCTC**** GCTCTATGCGAATACCTCCA	$(GAA)_{21}$	56	29/11	311–341	0.832/0.862	0.694
Sdu 23	DQ468097	**GCAGTGTGTGGCTATAAG**** GGTTGTTTCCTCCTTCAC	$(GACA)_8(GATA)_4$	50	29/15	240–328	0.870/0.897	0.550
Sdu 27	DQ468098	**CCTTCTGTCTTGACTCTGC***** CGATTCATCCAGCTTTAGG	$(GATA)_{13}$	56	29/8	266–298	0.783/0.630	0.060

GenBank Accession nos for clone sequences; Primer sequences are forward (top) and reverse (bottom); Repeat sequence indicates repeat motif; T_a is annealing temperature in °C; N is the number of individuals assayed; N_a is the number of alleles detected; Size range refers to alleles thus far uncovered; H_E and H_O are expected and observed heterozygosities, respectively; P_{HW} represents the probability of deviation from Hardy–Weinberg expectations. The fluorescently labelled primer (FAM*, HEX**, or NED***) is in bold.

*Dpn*II digests (21 × 384-well plates), were spotted in a 4 × 4 array onto 22.5 cm × 22.5 cm Hybond nylon membranes (Amersham), with each clone being spotted twice to eliminate false positives. Clones were fixed on membranes and probed four different times with a variety of (gamma) ^{32}P-labelled oligonucleotides: (i) one tetranucleotide cocktail with $(GATA)_9$, $(CATA)_8$ and $(GACA)_8$; and (ii) three individual trinucleotide probings with $(CAA)_8$, $(GAA)_8$ and $(TAA)_{13}$. A total of 86 positive clones were screened as follows. Plasmid DNA was isolated (alkaline lysis) with a BioRobot 8000 (QIAGEN). Miniprep DNA was quantified, normalized and both strands sequenced, using M13 forward and reverse sequencing primers and ABI PRISM BigDye Terminator version 3.1 (Applied Biosystems). Products were electrophoresed on an ABI PRISM 3100 DNA Genetic Analyser (Applied Biosystems). SEQUENCHER (Gene Codes Corporation) was used for DNA sequence base-calling and vector trimming. A total of 36 complete sequences containing microsatellite arrays were obtained from the positive clones. Twenty-eight primer pairs were designed flanking trinucleotide and tetranucleotide microsatellite arrays, using AMPLIFY 1.2 (Engels 1993) and NETPRIMER (www.premierbiosoft.com/netprimer).

Unlabelled PCR primers were purchased from Invitrogen and tested for amplification by screening two individuals obtained from John's Island, South Carolina. PCR

amplifications were performed with a PTC-200 thermocycler (MJ Research) in 10 μL-reaction volumes containing 100 ng DNA, 1 × PCR buffer (50 mm KCl, 10 mm Tris, 1% Triton-X 100), 0.1 U *Taq* DNA polymerase (Gibco-BRL), 0.5 μm of each primer, 200 μm of each dNTP, and 1 mm $MgCl_2$. PCR conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 38 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C–65 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. Once appropriate annealing temperatures were established for each primer pair, microsatellite arrays were tested for polymorphisms with an additional seven individuals from John's Island. A total of 18 microsatellite repeats were chosen for further screening (Table 1), consisting of 13 perfect (five tetranucleotides and eight trinucleotides) and five imperfect (three tetranucleotides, one trinucleotide and one combination dinucleotide/trinucleotide) repeat motifs. Length of cloned alleles ranged in size from 108 to 351 bp, and optimal annealing temperatures ranged from 50 °C to 60 °C (Table 1).

For further screening, one primer from each pair was labelled with one fluorescent label of Set D (Applied Biosystems): FAM, HEX, or NED. DNA was extracted from a total of 29 individuals from John's Island, South Carolina and PCR products were run on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems). Alleles were sized using the Genescan-500 ROX Size Standard (Applied Biosystems); allele sizing and calling were performed using GENESCAN 3.1.2 and GENOTYPER version 2.5 software. Genetic variability of the microsatellite markers was measured by the number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity. Fisher's exact test, as implemented in GDA (Lewis & Zaykin 2001), was used to test significance of departure from Hardy–Weinberg equilibrium (genotype) expectations at each microsatellite and departure from genotypic equilibrium at pairs of microsatellites.

Summary data are presented in Table 1. The number of alleles detected per microsatellite ranged from two (Sdu 9 and Sdu 11) to 20 (Sdu 21). Expected heterozygosity ranged from 0.068 (Sdu 8) to 0.950 (Sdu 21), whereas observed heterozygosity ranged from 0.069 (Sdu 8) to 0.966 (Sdu 10). Following Bonferroni correction (Rice 1989), genotypes at all 18 microsatellites fit expectations of Hardy–Weinberg equilibrium and all pairwise comparisons of microsatellites did not deviate significantly from genotypic equilibrium. The 18 microsatellites developed in this work will prove useful for studies of population genetics (e.g. stock structure, effective population size) of 'wild' greater amberjack populations and for breeding studies (e.g. assessment of genetic variability, inbreeding, parentage and QTL mapping) of domesticated populations.

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